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Authors	Lomax, Alan E.;Pradhananga, Sabindra;Sessenwein, Jessica L.;O'Malley, Dervla
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Bacterial modulation of visceral sensation: mediators and mechanisms

Alan E Lomax^{*1}, Sabindra Pradhananga¹, Jessica L Sessenwein¹, Dervla O'Malley^{2,3}

¹*Gastrointestinal Diseases Research Unit, Queen's University, Kingston, ON, Canada*

²*APC Microbiome Ireland, University College Cork, Ireland*

³*Department of Physiology, University College Cork, Ireland*

****Author for Correspondence:***

Alan Lomax

GIDRU Wing,

Kingston General Hospital,

Kingston ON K7L 2V7

Canada

Email: lomaxa@queensu.ca

25 **Abstract**

26 The potential role of the intestinal microbiota in modulating visceral pain has received increasing
27 attention during recent years. This has led to the identification of signaling pathways that have
28 been implicated in communication between gut bacteria and peripheral pain pathways. In
29 addition to the well-characterised impact of the microbiota on the immune system, which in turn
30 affects nociceptor excitability, bacteria can modulate visceral afferent pathways by effects on
31 enterocytes, enteroendocrine cells and the neurons themselves. Proteases produced by bacteria,
32 or by host cells in response to bacteria, can increase or decrease the excitability of nociceptive
33 dorsal root ganglion (DRG) neurons depending on the receptor activated. Short chain fatty acids
34 generated by colonic bacteria are involved in gut-brain communication, and intracolonic short
35 chain fatty acids have pro-nociceptive effects in rodents but may be anti-nociceptive in humans.
36 Gut bacteria modulate the synthesis and release of enteroendocrine cell mediators including
37 serotonin and glucagon-like peptide-1, which activate extrinsic afferent neurons. Deciphering the
38 complex interactions between visceral afferent neurons and the gut microbiota may lead to the
39 development of improved probiotic therapies for visceral pain.

40

Introduction

Visceral pain is a common and debilitating symptom of many digestive diseases, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) (17). Visceral pain is often resistant to conventional analgesics and can sometimes be exacerbated by opioid drugs (45, 55). In light of this, new therapeutics to relieve visceral pain are urgently needed. Progress towards this goal will be accelerated by a more complete understanding of the peripheral signaling molecules that modulate nociception in the gut.

The perception of pain is accomplished by neural pathways that connect the gut to the brain via the spinal cord. The first neurons in this chain have cell bodies in dorsal root ganglia (DRG), project sensory axons into the gut and form excitatory synapses in the dorsal horn of the spinal cord. A subpopulation of these neurons, called nociceptors, detects noxious stimuli and activates pain circuits in the brain. Host-derived mediators from biopsies of IBS and IBD patients induce hyperexcitability in nociceptive DRG neurons, leading to an exaggerated response to stimuli such as distension or a bowel movement (16, 26, 60). This change in nociceptor sensitivity is a major driver of visceral pain. Superimposed upon these peripheral changes are changes in central nervous system (CNS) circuits that amplify synaptic inputs from the periphery (17, 20). Thus, visceral pain results from a combination of peripheral sensitisation and central plasticity. Combating these pro-nociceptive influences are host-derived analgesic substances including endogenous opioids and cannabinoids (22, 124). This balance between pro-nociceptive and anti-nociceptive influences on DRG neuron excitability dictates the transmission of pain stimuli to the CNS and the perception of pain. Recent investigations have identified the gut microbiota as an additional factor in pain modulation, capable of either worsening or ameliorating pain (8, 88). Microbial modulation of visceral pain may have translational

relevance given the changes in microbiota composition associated with IBD and IBS. Although intestinal fungi may also play important roles in modulating visceral pain (21), in this review, we discuss the potential mediators of bacterial modulation of peripheral visceral pain pathways.

A potential role for gut bacteria in visceral pain signalling

The mutualistic relationship that has evolved between bacteria and eukaryotes includes the ability of commensal bacteria in the gut to influence behavior and pain (24, 40, 88, 96, 122). Although probiotics have been marketed for the treatment of visceral pain for over a decade, there is a lack of mechanistic insight into which bacteria, bacterial metabolites, or signaling pathways are most important. To date, much of the evidence in support of a role for the microbiota in regulating pain is derived from *in vivo* studies demonstrating that germ-free mice, or mice treated with antibiotics that alter the microbiota early in life, have heightened pain sensitivity (39-41, 74, 88, 90, 98). However, changes to pain sensitivity in germ-free mice may not be due solely to direct microbial-neuronal interaction, as germ-free mice exhibit a number of potentially confounding developmental changes to the immune system. Similarly, a study of visceral pain sensitivity in mice treated with a cocktail of antibiotics reported an increase in visceral pain accompanied by an increase in colonic myeloperoxidase activity, which is indicative of immune system activation (126). This suggested a role for inflammatory changes in nociceptive effects of modulating the microbiota. Although there is potential for bacterial products to directly activate nociceptive neurons, the evidence until recently, largely supported a role for epithelial and immune cells in mediating many of the effects of the gut microbiota on pain pathways *in vivo* (Table 1) (5, 80, 84, 131).

Bacteria as a source of host modulatory factors

There is a growing appreciation that the gut microbiota can be considered an endocrine organ, having the capability to directly or indirectly regulate different gastrointestinal and stress hormones, which may modify host physiological function (33). Intriguingly, the transfer of faecal matter from IBS patients is sufficient to evoke visceral hypersensitivity in gnotobiotic rats. This is not due to changes in mucosal permeability or immune activation, raising the possibility that bacterial metabolites in IBS patient stool directly modify gut-brain signalling (35). DRG neurons are capable of “sensing” the presence of microbes. They express functional microbial pattern recognition molecules, including toll like receptors and nucleotide-binding oligomerization domains 1 and 2 (91), whose activation can modulate neuronal excitability. Furthermore, the pathogenic bacterium *Staphylococcus aureus* directly excites DRG neurons through a toxin that forms cation-permeable pores in DRG neuronal membranes and through secretion of N-formylated peptides (32). In contrast to the pro-nociceptive effects of this skin pathogen however, the commensal gut microbes studied to date have inhibitory effects on DRG neuron excitability (88, 93, 109). Given the potential importance of the microbiota as a modulator of visceral pain, identification of the specific species involved and mediators responsible will be particularly important. Gut microbes produce a plethora of neuro-active compounds such as proteases (116), short chain fatty acids (SCFA) (99) and also classical neurotransmitters such as γ -amino butyric acid (GABA), dopamine and norepinephrine (94). We will consider the available evidence in support of a role for specific bacterial mediators in terms of their capability to directly access and act upon nerve circuits to modulate their function (39, 88, 137). We will also discuss microbe-mediated modulation of visceral pain pathways by using immune cells and enterocytes as cellular transducers (Figure 1).

Direct signalling by bacterial metabolites

Proteases

Extracellular proteases, in particular serine and cysteine proteases, are important modulators of visceral pain (127). Proteases are released from many eukaryotic cell types, including mast cells, neutrophils and enterocytes (97, 104). Recent *in vivo* and *in vitro* work has identified the gut microbiota as an important source of proteases (116) capable of affecting peripheral pain pathways (8, 81, 109). Pain regulation by proteases most often occurs through the activation of protease activated receptors (PARs). PARs are a family of four G-protein coupled receptors that lack conventional ligand binding sites and are instead activated via protease-mediated hydrolysis of amino acid residues. Upon protease cleavage, a tethered ligand within the receptor is revealed that activates intracellular signaling pathways (97). The net effect of receptor signaling depends not just on the PAR subtype involved but the specific amino acids hydrolysed (97). A consistent finding from numerous laboratories is that PAR-2 activation causes sustained hyperexcitability of DRG neurons (6, 34, 51, 136). Indeed, activation of nociceptor PAR-2 by mast cell tryptase and enterocyte derived trypsin-3 (85, 104) has been implicated in visceral pain (12, 63). However, nociceptive neurons also express PAR-1 and PAR-4. Activation of PAR-1 and PAR-4 reduces DRG neuron excitability and is anti-nociceptive (10, 11, 66, 104). PAR-2 activation *in vivo* by cysteine proteases in fecal supernatants from IBS patients enhanced the visceromotor response to colorectal distension in rats, an *in vivo* assay of visceral pain. In contrast, activation of PAR-4 by commensal microbes has an analgesic effect *in vivo* and *in vitro* (81, 109). The opposing effects of PAR-2, PAR-1 and -4 suggest that the balance between PAR-2, and PAR-1 - 4 activation could be a critical determinant of nociception.

While it seems clear that activation of PARs by proteases derived from the microbiota can modulate pain, an important unresolved issue is whether these proteases exert this influence

via actions on mucosal cells, immune cells or directly on DRG nerve terminals. The intestinal barrier is comprised of a mucus-coated epithelial monolayer whose integrity is maintained by tight junction proteins, which regulate the paracellular movement of luminal molecules. Beneath the epithelial layer, intrinsic and extrinsic neurons relay neural information both within the GI tract but also between the gut and the CNS. However, evidence that this communication system extends beyond the epithelial barrier to the microbially-dominated environment of the gut lumen, has resulted in it being referred to as the microbiota-gut-brain axis (19, 47, 76). It appears that at least in some circumstances, the impact of PAR activation on visceral pain is due to modulation of epithelial barrier function. Using a model of IBS in rodents, Miquel and colleagues found that proteases derived from *Faecalibacterium prausnitzii* inhibited the increase in visceral pain that results from neonatal maternal separation. In this case, the decrease in visceral pain was ascribed to PAR-4 mediated reversal of the increase in mucosal permeability in this model of visceral pain (81). Faecal supernatants from patients with chronic ulcerative colitis led to a decrease in visceromotor response to colorectal distention due to activation of PAR-4 (8). In a separate study, serine proteases from *Faecalibacterium prausnitzii* acted directly on nerve terminals to inhibit colonic sensory nerve spike discharge and reduced the excitability of colon-projecting DRG neurons via PAR-4 activation (109). Furthermore, these proteases reversed DRG neuronal hyperexcitability caused by the dextran sulphate sodium model of colitis in mice (109).

Opposite findings have been reported for microbial activation of PAR-2. Luminal administration of faecal supernatants from patients with diarrhea-predominant IBS increased visceral pain sensitivity and impaired mucosal barrier function *in vivo* via PAR-2 activation (49). Consistent with the ability of luminal proteases to have pronociceptive effects, luminal administration of the PAR-2 activating serine protease, cathepsin S, was sufficient to increase

visceromotor responses in mice in a PAR-2-dependent manner (27). Similarly, activation of PAR-2 by host derived proteases causes a sustained increase in the excitability of mouse DRG neurons (67). Thus, although there is abundant evidence that activation of neuronal PAR-2 has pro-nociceptive effects, it remains unclear whether neuronal PAR-2, in addition to mucosal PAR-2, participates in the pro-nociceptive effects of bacterial proteases. Cell-specific receptor knockout strategies will be important tools in identifying which PAR-expressing cells are most important to visceral pain modulation *in vivo*.

In addition to microbial-derived proteases, the microbiota is a rich source of protease inhibitors (54) including siropins, which has been shown to mitigate the effect of host-derived proteases implicated in IBD pathogenesis (82). A recent study using a rodent model of post-inflammatory hypersensitivity provided valuable evidence that synthetic protease inhibitors can mitigate the pro-nociceptive effects of proteases in this model (28). It therefore appears that the balance between the activity of proteases and protease inhibitors can influence visceral perception and may be an important target for novel therapeutics (128).

Short chain fatty acids

Short chain fatty acids (SCFAs) are produced by the fermentation of dietary polysaccharides that are metabolized by the anaerobic bacteria found in the cecum and colon. Formate, acetate, butyrate, and propionate are the major byproducts of this fermentation process (83). Earlier reports have identified *Fecalibacterium prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii* and *Roseburia faecis* as bacteria capable of producing butyrate. Likewise, acetate and pyruvate are produced by enteric bacteria such as *Blautia hydrogenotrophica*; propionate, on the other hand, can be produced by *Bacteroidetes* and *Firmicutes* (72).

A well-established effect of butyrate is inhibition of bowel inflammation and enhancement of mucosal repair, which would have an indirect effect on inflammatory visceral pain (103). SCFAs also modulate the enteric nervous system (113) and have been posited as an important mediator of microbiota-gut-brain communication (88). Microbial dysbiosis, due to the administration of antibiotics or due to modulation of diet, led to a decrease in SCFA and an increase in visceral sensitivity (38, 90, 100, 112). This suggests an association between SCFA and visceral pain modulation but does not directly establish a causal relationship. Contrary to these studies, when SCFAs were administered to control rats and rats with TNBS-induced colitis, visceral hypersensitivity was not improved by any of the SCFAs (acetate, propionate and butyrate) used (121). In fact, butyrate administration decreased the noxious pressure threshold in rats, indicating a pronociceptive effect; this phenomenon was more pronounced in control rats than in TNBS- treated rats. This observation is supported by a report that rectal administration of sodium butyrate induced colonic hypersensitivity in rats (133). This pronociceptive effect was associated with neuronal activation of extracellular signal related kinase (ERK)1/2 and an enhancement of DRG neuronal excitability. However, a study of healthy human volunteers concluded that butyrate treatment induced a dose-dependent reduction of visceral sensitivity (125). In summary, despite evidence implicating SCFAs in mediating gut-brain communication in general, there are conflicting findings regarding the role of SCFAs in modulating visceral pain.

Microbial neurotransmitters and neurotrophic factors

Microbial depletion and recolonization studies have linked microbial modification of neuroactive compounds in the gut-brain communication axis to diseases of the peripheral and central nervous system (119). Germ-free studies illustrate the crucial role of microbes in the development of

brain function and expression of central neurochemicals (15, 23) however, antibiotic treatment in mature animals can avoid the confounding developmental effects of early-life microbial alterations. Hoban and colleagues reported modification of central monoamines, serotonin and brain derived neurotrophic factor (BDNF) following sustained antibiotic administration to adult rats. These changes were accompanied by altered behaviors and diminished visceral pain sensitivity to colorectal distension (58). Interestingly, antibiotic-related alterations in neurotransmitters can be long-lasting and have different functional outcomes when administered early in life. A gender-specific increase in visceral sensitivity, which was linked to decreases in spinal cord expression of transient receptor potential (TRP)V1, α 2A adrenergic receptors and cholecystokinin B receptors, was noted in male rats treated with vancomycin from postnatal days 4-13 (90).

In addition to modification of host neurotransmitters, microbes also exhibit the capacity to secrete functional neurotransmitters and neurotrophins. GABA, the major inhibitory neurotransmitter, is synthesized by several *Lactobacilli* and *Bifidobacteria* (14, 129). As GABA receptor agonists can suppress visceral pain responses to colorectal distension (56) and inflammation-induced pain signals (73), this may contribute to nociceptive signaling from the gut (62). Dopamine and norepinephrine, which have reported anti-nociceptive effects of visceral pain sensitivity (37, 92), are also produced by several gut bacterial species, including *Bacilli* and *Escherichia* (94, 129). BDNF, an important neurotrophic regulator of synaptic plasticity and neurogenesis, is purported to be a hallmark of altered microbiota-gut-brain axis signaling, given that its expression is altered in germ-free mice (87, 120) and in antibiotic- (58) and prebiotic-treated mice (107). Moreover, BDNF is expressed on TRPV1-expressing nociceptive DRG neurons (132) and neutralizing BDNF blocked visceral hypersensitivity in inflammatory colonic

hypersensitivity (42). In IBS patients, increased expression of nerve growth factor (NGF) correlated with visceral pain sensitivity (134), which may be due to sensitization of pro-nociceptive receptors on primary afferent neurons. Indeed, NGF increases TRPV1 expression in DRGs (110). In the context of microbial modification of host molecules, an *in vitro* study demonstrated that *Lactobacillus rhamnosus* induces anti-inflammatory effects in human epithelial cells which is mediated by NGF (75). Although intriguing, evidence that gut bacteria have the capacity to secrete neurotransmitters and neurotrophins, does not explain how neuromodulatory molecules in the external environment of the gut lumen can modify gut-to-brain nociceptive signalling. As afferent nerves do not reach through the epithelium into the gut lumen, further mechanistic studies are needed to determine how bacterially-derived neuromodulatory factors can cross the gut barrier to influence gut-brain signalling.

Indirect signaling

Serotonin secretion from Enterochromaffin cells

Serotonin has long been recognised as a critical regulator of gut function, inflammation and pain (50, 77). Accordingly, the release of serotonin from enterochromaffin (EC) cells and its sites of action are important therapeutic targets for visceral pain. Two recent independent reports delineated the ability of microbes to modulate serotonin synthesis by EC cells. One study reported an increase in serotonin production in mice colonised with human fecal microbiota, compared to germ-free mice (99). This was associated with an increase in expression of tryptophan hydroxylase 1 (TPH1), the rate limiting enzyme for serotonin synthesis in EC cells. Consistent with the ability of microbial metabolites to increase TPH1 expression, the SCFAs, sodium acetate and sodium butyrate, increased TPH1 expression in a human-derived EC cell

line. The second study identified spore-forming bacteria as important modulators of serotonin production by EC cells, and revealed that this effect occurred in the colon but not the small intestine (135). Furthermore, EC cell serotonin modulation by microbiota was also observed in RAG1 knockout mice which lack T and B cells, suggesting a direct action on EC cells rather than an indirect effect via immunomodulation. SCFAs were also implicated as modulators of EC cell function, which may be an important mechanism of pain modulation by microbiota. Other bacterial metabolites, such as bile acids and p-aminobenzoate, have also been implicated in regulating serotonin production. From these findings it appears that several bacterial signaling pathways depend on the release of serotonin from EC cell as a means of modulating gut function, inflammation and visceral pain. In addition to microbial modulation of serotonin release, Kwon and colleagues have recently (69) demonstrated that host-derived serotonin has direct and species-specific effects on the growth of commensal microbes *in vivo* and *in vitro*. Furthermore, the secretion of the anti-microbial peptide α -defensin from the HT-29 epithelial cell line was inhibited by serotonin (69). These findings illustrate the complex and bidirectional nature of the interactions between gut microbes and enterochromaffin cells.

GLP-1 secretion from L-cells

Similar to EC cells, GLP-1-secreting L-cells may act as chemosensory sentinels, conveying information about the luminal environment to the host. L-cells are polarised, electrically excitable enteroendocrine cells (31), which sense the arrival of nutrients, such as glucose and amino acids, in the small intestine. Despite the reduced probability of nutrients being present, the abundance of GLP-1-secreting L-cells increases towards the distal end of the GI tract (117). Consistent with the contents of the colonic lumen, L-cells in this region express receptors for SCFAs and bile acids (101, 123). Moreover, dietary supplementation with SCFAs (123), the

introduction of specific commensal strains (9, 118) or antibiotic treatment (61) increased GLP-1 levels. Somewhat counter-intuitively, one study determined that serum GLP-1 was also elevated in germ-free mice (108), although other researchers found that germ-free mice exhibited a strong state of GLP-1 resistance, with impaired GLP-1 evoked gut-brain signalling and enteric nervous system function (52). A clinical trial in IBS patients found that administration of a GLP-1 mimetic reduced acute abdominal pain in patients (57). GLP-1 can act as a classical endocrine hormone, however GLP-1 also has direct neurostimulatory actions on vagal afferent neurons (78). Furthermore, there is evidence of direct, physical contact between a pseudopod-like elongation of L-cells and afferent nerve fibres (18), providing for a potential neural signalling pathway in the modification of GI function. Thus, L-cells are appropriately positioned to facilitate cross-barrier signalling from the gut lumen to the host peripheral nervous system and on to the CNS, and should be investigated as a potential modulator of visceral pain.

Histamine release from mast cells

Histamine, which is mainly secreted by mast cells, promotes allergic inflammation but also appears to play a role in visceral nociception. Indeed, histamine-containing secretions from IBS patient mucosal mast cells have been shown to excite rat nociceptive visceral afferent nerves, and are thus likely to participate in relaying visceral pain signals (13). Of the four histamine receptor subtypes, H1R and H2R are most prevalent in the gut. Similar to the opposing actions of PAR subtypes described earlier, activation of H1R promotes pro-inflammatory pathways (30), whereas H2R suppresses inflammation (111). In patients with IBD, reduced expression of H2R may underlie decreased suppression of TLR-induced cytokine secretion in this patient population (111). H1R antagonists decreased abdominal pain in IBS patients (68) and in a rat model of visceral hypersensitivity (115). Moreover, IBS patient biopsies display increased expression of

H1R (106). Histamine may also be secreted by bacterial species such as *Lactobacillus reuteri* 6475, a commonly-used probiotic (114), which can reduce intestinal inflammation (48) and may also have an impact of visceral pain sensitivity.

Vagal afferent pathways

Vagal afferent neurons may also participate in the sensory arm of gut-brain nociceptive signaling. Although electrical stimulation of abdominal vagal afferents does not induce pain *per se*, nociceptive signaling may be modulated by vagal activity (7). Vagal nerve activation may in fact, induce an inhibitory modulation of chemically or mechanically-provoked insults (29, 53), as noted in a rat model of visceral pain where vagal nerve stimulation had an anti-nociceptive effect (138). Vagal afferent terminals are located within enteric ganglia, and in the smooth muscle and mucosal layers, where they are well-positioned to sense chemo-nociceptive signals (70, 95, 130). Given the essential role of the vagus nerve in mediating microbe-gut-brain communication (15, 23), future work should address whether modulation of vagal afferent pathways by bacteria impacts visceral pain.

Conclusions

There is abundant evidence that the microbiota is capable of modifying visceral pain *in vivo*. However, clinical trials of probiotics as therapies for visceral pain have yielded equivocal results. This may reflect patient heterogeneity, patient compliance, or the variety of probiotic formulations used, which in turn reflects a relative paucity of mechanistic work identifying the most important microbial species and mediators to target for clinical benefit. A number of issues remain unresolved in bridging the gaps between our present state of knowledge and successful manipulation of the gut microbiota to alleviate pain. For example, the detection of high threshold noxious stimuli in rodents is accomplished by visceral afferent neurons with terminals

that lie along serosal and mesenteric blood vessels (25). Furthermore, based on a limited number of recordings from visceral afferent neurons from human bowel, the majority of afferent terminals that have been characterized to date have been located in the muscle and vasculature. Thus, it appears that luminal mediators from the microbiota may have traverse the epithelial barrier and enter the circulation to access and modulate gut nociceptive terminals. Future studies of full-thickness resected bowel preparations from patients may provide insight into how the luminal microbiota accesses these terminals. Another potential caveat when translating findings from rodents to patients is that signaling mechanisms that are inhibitory in rodents may be excitatory in patients, and vice versa. A recent Ca^{2+} imaging study of PAR activation in human DRG neurons reported that PAR-1 activation in human neurons is excitatory (43), whereas PAR-1 is inhibitory in rodents (10). By increasing mechanistic insights into the interplay between the microbiota and peripheral pain pathways, particularly using patient microbiota and human DRG neurons (59), improved therapies that harness the analgesic properties of the microbiota may soon be on the horizon.

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332 References

- 333 1. **Agostini S, Gouben M, Tondereau V, Salvador-Cartier C, Bezirard V, Leveque M,**
334 **Keranen H, Theodorou V, Bourdu-Naturel S, Goupil-Feuillerat N, Legrain-Raspaud S, and**
335 **Eutamene H.** A marketed fermented dairy product containing *Bifidobacterium lactis* CNCM I-
336 2494 suppresses gut hypersensitivity and colonic barrier disruption induced by acute stress in
337 rats. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal*
338 *Motility Society* 24: 376-e172, 2012.
- 339 2. **Ait-Belgnaoui A, Eutamene H, Houdeau E, Bueno L, Fioramonti J, and Theodorou V.**
340 *Lactobacillus farciminis* treatment attenuates stress-induced overexpression of Fos protein in
341 spinal and supraspinal sites after colorectal distension in rats. *Neurogastroenterology and*
342 *motility : the official journal of the European Gastrointestinal Motility Society* 21: 567-573,
343 e518-569, 2009.
- 344 3. **Ait-Belgnaoui A, Han W, Lamine F, Eutamene H, Fioramonti J, Bueno L, and Theodorou**
345 **V.** *Lactobacillus farciminis* treatment suppresses stress induced visceral hypersensitivity: a
346 possible action through interaction with epithelial cell cytoskeleton contraction. *Gut* 55: 1090-
347 1094, 2006.
- 348 4. **Ait-Belgnaoui A, Payard I, Rolland C, Harkat C, Braniste V, Theodorou V, and Tompkins**
349 **TA.** *Bifidobacterium longum* and *Lactobacillus helveticus* Synergistically Suppress Stress-related
350 Visceral Hypersensitivity Through Hypothalamic-Pituitary-Adrenal Axis Modulation. *J*
351 *Neurogastroenterol Motil* 24: 138-146, 2018.
- 352 5. **Al-Nedawi K, Mian MF, Hossain N, Karimi K, Mao Y, Forsythe P, Min KK, Stanisz AM,**
353 **Kunze WA, and Bienenstock J.** Gut commensal microvesicles reproduce parent bacterial signals
354 to host immune and enteric nervous systems. *FASEB J*, 2014.
- 355 6. **Amadesi S, Cottrell GS, Divino L, Chapman K, Grady EF, Bautista F, Karanjia R, Barajas-**
356 **Lopez C, Vanner S, Vergnolle N, and Bunnett NW.** Protease-activated receptor 2 sensitizes
357 TRPV1 by protein kinase C epsilon- and A-dependent mechanisms in rats and mice. *The Journal*
358 *of physiology* 575: 555-571, 2006.
- 359 7. **Andrews PL and Sanger GJ.** Abdominal vagal afferent neurones: an important target for
360 the treatment of gastrointestinal dysfunction. *Current opinion in pharmacology* 2: 650-656,
361 2002.
- 362 8. **Annahazi A, Gecse K, Dabek M, Ait-Belgnaoui A, Rosztoczy A, Roka R, Molnar T,**
363 **Theodorou V, Wittmann T, Bueno L, and Eutamene H.** Fecal proteases from diarrheic-IBS and
364 ulcerative colitis patients exert opposite effect on visceral sensitivity in mice. *Pain* 144: 209-217,
365 2009.
- 366 9. **Aoki R, Kamikado K, Suda W, Takii H, Mikami Y, Suganuma N, Hattori M, and Koga Y.** A
367 proliferative probiotic *Bifidobacterium* strain in the gut ameliorates progression of metabolic
368 disorders via microbiota modulation and acetate elevation. *Sci Rep* 7: 43522, 2017.
- 369 10. **Asfaha S, Brussee V, Chapman K, Zochodne DW, and Vergnolle N.** Proteinase-activated
370 receptor-1 agonists attenuate nociception in response to noxious stimuli. *British journal of*
371 *pharmacology* 135: 1101-1106, 2002.

11. **Auge C, Balz-Hara D, Steinhoff M, Vergnolle N, and Cenac N.** Protease-activated receptor-4 (PAR 4): a role as inhibitor of visceral pain and hypersensitivity. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 21: 1189-e1107, 2009.
12. **Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, and Corinaldesi R.** Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 126: 693-702, 2004.
13. **Barbara G, Wang B, Stanghellini V, de Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, and Corinaldesi R.** Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 132: 26-37, 2007.
14. **Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, and Stanton C.** gamma-Aminobutyric acid production by culturable bacteria from the human intestine. *J Appl Microbiol* 113: 411-417, 2012.
15. **Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, Deng Y, Blennerhassett PA, Fahnestock M, Moine D, Berger B, Huizinga JD, Kunze W, McLean PG, Bergonzelli GE, Collins SM, and Verdu EF.** The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 23: 1132-1139, 2011.
16. **Beyak MJ and Vanner S.** Inflammation-induced hyperexcitability of nociceptive gastrointestinal DRG neurones: the role of voltage-gated ion channels. *NeurogastroenterolMotil* 17: 175-186, 2005.
17. **Bielefeldt K, Davis B, and Binion DG.** Pain and inflammatory bowel disease. *Inflammatory bowel diseases* 15: 778-788, 2009.
18. **Bohorquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, Wang F, and Liddle RA.** Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest* 125: 782-786, 2015.
19. **Bonaz B, Bazin T, and Pellissier S.** The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. *Front Neurosci* 12: 49, 2018.
20. **Bonaz BL and Bernstein CN.** Brain-gut interactions in inflammatory bowel disease. *Gastroenterology* 144: 36-49, 2013.
21. **Botschuijver S, Roeselers G, Levin E, Jonkers DM, Welting O, Heinsbroek SEM, de Weerd HH, Boekhout T, Fornai M, Masclee AA, Schuren FHJ, de Jonge WJ, Seppen J, and van den Wijngaard RM.** Intestinal Fungal Dysbiosis Is Associated With Visceral Hypersensitivity in Patients With Irritable Bowel Syndrome and Rats. *Gastroenterology* 153: 1026-1039, 2017.
22. **Boue J, Basso L, Cenac N, Blanpied C, Rolli-Derkinderen M, Neunlist M, Vergnolle N, and Dietrich G.** Endogenous regulation of visceral pain via production of opioids by colitogenic CD4(+) T cells in mice. *Gastroenterology* 146: 166-175, 2014.
23. **Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, and Cryan JF.** Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 108: 16050-16055, 2011.

24. **Bravo JA, Julio-Pieper M, Forsythe P, Kunze W, Dinan TG, Bienenstock J, and Cryan JF.** Communication between gastrointestinal bacteria and the nervous system. *Current opinion in pharmacology* 12: 667-672, 2012.
25. **Brierley SM, Hibberd TJ, and Spencer NJ.** Spinal Afferent Innervation of the Colon and Rectum. *Front Cell Neurosci* 12: 467, 2018.
26. **Brierley SM and Linden DR.** Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nature reviews Gastroenterology & hepatology* 11: 611-627, 2014.
27. **Cattaruzza F, Lyo V, Jones E, Pham D, Hawkins J, Kirkwood K, Valdez-Morales E, Ibeakanma C, Vanner SJ, Bogyo M, and Bunnett NW.** Cathepsin S is activated during colitis and causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. *Gastroenterology* 141: 1864-1874 e1861-1863, 2011.
28. **Ceuleers H, Hanning N, Heirbaut J, Van Remoortel S, Joossens J, Van Der Veken P, Francque SM, De Bruyn M, Lambeir AM, De Man JG, Timmermans JP, Augustyns K, De Meester I, and De Winter BY.** Newly developed serine protease inhibitors decrease visceral hypersensitivity in a post-inflammatory rat model for irritable bowel syndrome. *British journal of pharmacology* 175: 3516-3533, 2018.
29. **Chen SL, Wu XY, Cao ZJ, Fan J, Wang M, Owyang C, and Li Y.** Subdiaphragmatic vagal afferent nerves modulate visceral pain. *American journal of physiology* 294: G1441-1449, 2008.
30. **Chen X, Egly C, Riley AM, Li W, Tewson P, Hughes TE, Quinn AM, and Obukhov AG.** PKC-dependent Phosphorylation of the H1 Histamine Receptor Modulates TRPC6 Activity. *Cells* 3: 247-257, 2014.
31. **Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, and Reimann F.** Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep* 9: 1202-1208, 2014.
32. **Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR, Bubeck Wardenburg J, Hwang SW, Carroll MC, and Woolf CJ.** Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* 501: 52-57, 2013.
33. **Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, and Dinan TG.** Minireview: Gut microbiota: the neglected endocrine organ. *Mol Endocrinol* 28: 1221-1238, 2014.
34. **Coelho AM, Vergnolle N, Guiard B, Fioramonti J, and Bueno L.** Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. *Gastroenterology* 122: 1035-1047, 2002.
35. **Crouzet L, Gaultier E, Del'Homme C, Cartier C, Delmas E, Dapoigny M, Fioramonti J, and Bernalier-Donadille A.** The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil* 25: e272-282, 2013.
36. **Dai C, Guandalini S, Zhao DH, and Jiang M.** Antinociceptive effect of VSL#3 on visceral hypersensitivity in a rat model of irritable bowel syndrome: a possible action through nitric oxide pathway and enhance barrier function. *Mol Cell Biochem* 362: 43-53, 2012.
37. **Danzebrink RM and Gebhart GF.** Antinociceptive effects of intrathecal adrenoceptor agonists in a rat model of visceral nociception. *J Pharmacol Exp Ther* 253: 698-705, 1990.
38. **De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, and Lionetti P.** Impact of diet in shaping gut microbiota revealed by a comparative

study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* 107: 14691-14696, 2010.

39. **De Palma G, Blennerhassett P, Lu J, Deng Y, Park AJ, Green W, Denou E, Silva MA, Santacruz A, Sanz Y, Surette MG, Verdu EF, Collins SM, and Bercik P.** Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature communications* 6: 7735, 2015.

40. **De Palma G, Collins SM, and Bercik P.** The microbiota-gut-brain axis in functional gastrointestinal disorders. *Gut microbes* 5: 419-429, 2014.

41. **De Palma G, Lynch MD, Lu J, Dang VT, Deng Y, Jury J, Umeh G, Miranda PM, Pigrau Pastor M, Sidani S, Pinto-Sanchez MI, Philip V, McLean PG, Hagelsieb MG, Surette MG, Bergonzelli GE, Verdu EF, Britz-McKibbin P, Neufeld JD, Collins SM, and Bercik P.** Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. *Science translational medicine* 9, 2017.

42. **Delafooy L, Gelot A, Ardid D, Eschaliere A, Bertrand C, Doherty AM, and Diop L.** Interactive involvement of brain derived neurotrophic factor, nerve growth factor, and calcitonin gene related peptide in colonic hypersensitivity in the rat. *Gut* 55: 940-945, 2006.

43. **Desormeaux C, Bautzova T, Garcia-Caraballo S, Rolland C, Barbaro MR, Brierley SM, Barbara G, Vergnolle N, and Cenac N.** Protease-activated receptor 1 is implicated in irritable bowel syndrome mediators-induced signaling to thoracic human sensory neurons. *Pain* 159: 1257-1267, 2018.

44. **Distrutti E, Cipriani S, Mencarelli A, Renga B, and Fiorucci S.** Probiotics VSL#3 protect against development of visceral pain in murine model of irritable bowel syndrome. *PloS one* 8: e63893, 2013.

45. **Drossman DA, Morris CB, Edwards H, Wrennall CE, Weinland SR, Aderoju AO, Kulkarni-Kelapure RR, Hu YJ, Dalton C, Bouma MH, Zimmerman J, Rooker C, Leserman J, and Bangdiwala SI.** Diagnosis, characterization, and 3-month outcome after detoxification of 39 patients with narcotic bowel syndrome. *The American journal of gastroenterology* 107: 1426-1440, 2012.

46. **Eutamene H, Lamine F, Chabo C, Theodorou V, Rochat F, Bergonzelli GE, Cortesy-Theulaz I, Fioramonti J, and Bueno L.** Synergy between *Lactobacillus paracasei* and its bacterial products to counteract stress-induced gut permeability and sensitivity increase in rats. *The Journal of nutrition* 137: 1901-1907, 2007.

47. **Forsythe P, Bienenstock J, and Kunze WA.** Vagal pathways for microbiome-brain-gut axis communication. *Advances in experimental medicine and biology* 817: 115-133, 2014.

48. **Ganesh BP, Hall A, Ayyaswamy S, Nelson JW, Fultz R, Major A, Haag A, Esparza M, Lugo M, Venable S, Whary M, Fox JG, and Versalovic J.** Diacylglycerol kinase synthesized by commensal *Lactobacillus reuteri* diminishes protein kinase C phosphorylation and histamine-mediated signaling in the mammalian intestinal epithelium. *Mucosal Immunol* 11: 380-393, 2018.

49. **Gecse K, Roka R, Ferrier L, Leveque M, Eutamene H, Cartier C, Ait-Belgnaoui A, Rosztoczy A, Izbeki F, Fioramonti J, Wittmann T, and Bueno L.** Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic luminal factor impairing colonic permeability and sensitivity. *Gut* 57: 591-599, 2008.

50. **Gershon MD.** Serotonin is a sword and a shield of the bowel: serotonin plays offense and defense. *Transactions of the American Clinical and Climatological Association* 123: 268-280; discussion 280, 2012.
51. **Grant AD, Cottrell GS, Amadesi S, Trevisani M, Nicoletti P, Materazzi S, Altier C, Cenac N, Zamponi GW, Bautista-Cruz F, Lopez CB, Joseph EK, Levine JD, Liedtke W, Vanner S, Vergnolle N, Geppetti P, and Bunnett NW.** Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. *The Journal of physiology* 578: 715-733, 2007.
52. **Grasset E, Puel A, Charpentier J, Collet X, Christensen JE, Terce F, and Burcelin R.** A Specific Gut Microbiota Dysbiosis of Type 2 Diabetic Mice Induces GLP-1 Resistance through an Enteric NO-Dependent and Gut-Brain Axis Mechanism. *Cell metabolism* 25: 1075-1090 e1075, 2017.
53. **Gschossmann JM, Mayer EA, Miller JC, and Raybould HE.** Subdiaphragmatic vagal afferent innervation in activation of an opioidergic antinociceptive system in response to colorectal distension in rats. *Neurogastroenterol Motil* 14: 403-408, 2002.
54. **Guo CJ, Chang FY, Wyche TP, Backus KM, Acker TM, Funabashi M, Taketani M, Donia MS, Nayfach S, Pollard KS, Craik CS, Cravatt BF, Clardy J, Voigt CA, and Fischbach MA.** Discovery of Reactive Microbiota-Derived Metabolites that Inhibit Host Proteases. *Cell* 168: 517-526 e518, 2017.
55. **Hanson KA, Loftus EV, Jr., Harmsen WS, Diehl NN, Zinsmeister AR, and Sandborn WJ.** Clinical features and outcome of patients with inflammatory bowel disease who use narcotics: a case-control study. *Inflammatory bowel diseases* 15: 772-777, 2009.
56. **Hara K, Saito Y, Kirihaara Y, Yamada Y, Sakura S, and Kosaka Y.** The interaction of antinociceptive effects of morphine and GABA receptor agonists within the rat spinal cord. *Anesth Analg* 89: 422-427, 1999.
57. **Hellstrom PM, Hein J, Bytzer P, Bjornsson E, Kristensen J, and Schambye H.** Clinical trial: the glucagon-like peptide-1 analogue ROSE-010 for management of acute pain in patients with irritable bowel syndrome: a randomized, placebo-controlled, double-blind study. *Aliment Pharmacol Ther* 29: 198-206, 2009.
58. **Hoban AE, Moloney RD, Golubeva AV, McVey Neufeld KA, O'Sullivan O, Patterson E, Stanton C, Dinan TG, Clarke G, and Cryan JF.** Behavioural and neurochemical consequences of chronic gut microbiota depletion during adulthood in the rat. *Neuroscience* 339: 463-477, 2016.
59. **Hockley JRF, Smith ESJ, and Bulmer DC.** Human visceral nociception: findings from translational studies in human tissue. *Am J Physiol Gastrointest Liver Physiol* 315: G464-G472, 2018.
60. **Hughes P, Brierly S, and Blackshaw LA.** Post inflammatory modification of colonic afferent mechanosensitivity. *ClinExpPharmacolPhysiol*, 2009.
61. **Hwang I, Park YJ, Kim YR, Kim YN, Ka S, Lee HY, Seong JK, Seok YJ, and Kim JB.** Alteration of gut microbiota by vancomycin and bacitracin improves insulin resistance via glucagon-like peptide 1 in diet-induced obesity. *FASEB J* 29: 2397-2411, 2015.
62. **Hyland NP and Cryan JF.** A Gut Feeling about GABA: Focus on GABA(B) Receptors. *Frontiers in pharmacology* 1: 124, 2010.
63. **Ibeakanma C, Ochoa-Cortes F, Miranda-Morales M, McDonald T, Spreadbury I, Cenac N, Cattaruzza F, Hurlbut D, Vanner S, Bunnett N, Vergnolle N, and Vanner S.** Brain-gut

interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. *Gastroenterology* 141: 2098-2108 e2095, 2011.

64. **Johnson AC, Greenwood-Van Meerveld B, and McRorie J.** Effects of Bifidobacterium infantis 35624 on post-inflammatory visceral hypersensitivity in the rat. *Digestive diseases and sciences* 56: 3179-3186, 2011.

65. **Kannampalli P, Pochiraju S, Chichlowski M, Berg BM, Rudolph C, Bruckert M, Miranda A, and Sengupta JN.** Probiotic Lactobacillus rhamnosus GG (LGG) and prebiotic prevent neonatal inflammation-induced visceral hypersensitivity in adult rats. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 26: 1694-1704, 2014.

66. **Karanja R, Spreadbury I, Bautista-Cruz F, Tsang ME, and Vanner S.** Activation of protease-activated receptor-4 inhibits the intrinsic excitability of colonic dorsal root ganglia neurons. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 21: 1218-1221, 2009.

67. **Kayssi A, Amadesi S, Bautista F, Bunnett NW, and Vanner S.** Mechanisms of protease-activated receptor 2-evoked hyperexcitability of nociceptive neurons innervating the mouse colon. *J Physiol* 580: 977-991, 2007.

68. **Klooker TK, Braak B, Koopman KE, Welting O, Wouters MM, van der Heide S, Schemann M, Bischoff SC, van den Wijngaard RM, and Boeckstaens GE.** The mast cell stabiliser ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut* 59: 1213-1221, 2010.

69. **Kwon YH, Wang H, Denou E, Ghia JE, Rossi L, Fontes ME, Bernier SP, Shajib MS, Banskota S, Collins SM, Surette MG, and Khan WI.** Modulation of Gut Microbiota Composition by Serotonin Signaling Influences Intestinal Immune Response and Susceptibility to Colitis. *Cellular and molecular gastroenterology and hepatology* 7: 709-728, 2019.

70. **Lamb K, Kang YM, Gebhart GF, and Bielefeldt K.** Gastric inflammation triggers hypersensitivity to acid in awake rats. *Gastroenterology* 125: 1410-1418, 2003.

71. **Li YJ, Dai C, and Jiang M.** Mechanisms of Probiotic VSL#3 in a Rat Model of Visceral Hypersensitivity Involves the Mast Cell-PAR2-TRPV1 Pathway. *Digestive diseases and sciences* 64: 1182-1192, 2019.

72. **Louis P, Hold GL, and Flint HJ.** The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 12: 661-672, 2014.

73. **Lu Y and Westlund KN.** Effects of baclofen on colon inflammation-induced Fos, CGRP and SP expression in spinal cord and brainstem. *Brain Res* 889: 118-130, 2001.

74. **Luczynski P, Tramullas M, Viola M, Shanahan F, Clarke G, O'Mahony S, Dinan TG, and Cryan JF.** Microbiota regulates visceral pain in the mouse. *eLife* 6, 2017.

75. **Ma D, Forsythe P, and Bienenstock J.** Live Lactobacillus rhamnosus [corrected] is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. *Infect Immun* 72: 5308-5314, 2004.

76. **Martin CR, Osadchiy V, Kalani A, and Mayer EA.** The Brain-Gut-Microbiome Axis. *Cell Mol Gastroenterol Hepatol* 6: 133-148, 2018.

77. **Mawe GM and Hoffman JM.** Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. *Nature reviews Gastroenterology & hepatology* 10: 473-486, 2013.

78. **McKee DP and Quigley EM.** Intestinal motility in irritable bowel syndrome: is IBS a motility disorder? Part 1. Definition of IBS and colonic motility. *Digestive diseases and sciences* 38: 1761-1772, 1993.
79. **McKernan DP, Fitzgerald P, Dinan TG, and Cryan JF.** The probiotic Bifidobacterium infantis 35624 displays visceral antinociceptive effects in the rat. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 22: 1029-1035, e1268, 2010.
80. **McVey Neufeld KA, Mao YK, Bienenstock J, Foster JA, and Kunze WA.** The microbiome is essential for normal gut intrinsic primary afferent neuron excitability in the mouse. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 25: 183-e188, 2013.
81. **Miquel S, Martin R, Lashermes A, Gillet M, Meleine M, Gelot A, Eschaliere A, Ardid D, Bermudez-Humaran LG, Sokol H, Thomas M, Theodorou V, Langella P, and Carvalho FA.** Antinociceptive effect of Faecalibacterium prausnitzii in non-inflammatory IBS-like models. *Sci Rep* 6: 19399, 2016.
82. **Mkaouer H, Akermi N, Mariaule V, Boudebouze S, Gaci N, Szukala F, Pons N, Marquez J, Gargouri A, Maguin E, and Rhimi M.** Siropins, novel serine protease inhibitors from gut microbiota acting on human proteases involved in inflammatory bowel diseases. *Microb Cell Fact* 15: 201, 2016.
83. **Morrison DJ and Preston T.** Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* 7: 189-200, 2016.
84. **Muller PA, Koscsó B, Rajani GM, Stevanovic K, Berres ML, Hashimoto D, Mortha A, Leboeuf M, Li XM, Mucida D, Stanley ER, Dahan S, Margolis KG, Gershon MD, Merad M, and Bogunovic M.** Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. *Cell* 158: 300-313, 2014.
85. **Nasser Y, Boeckxstaens GE, Wouters MM, Schemann M, and Vanner S.** Using human intestinal biopsies to study the pathogenesis of irritable bowel syndrome. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 26: 455-469, 2014.
86. **Nebot-Vivinus M, Harkat C, Bziouche H, Cartier C, Plichon-Dainese R, Moussa L, Eutamene H, Pishvaie D, Holowacz S, Seyrig C, Piche T, and Theodorou V.** Multispecies probiotic protects gut barrier function in experimental models. *World J Gastroenterol* 20: 6832-6843, 2014.
87. **Neufeld KM, Kang N, Bienenstock J, and Foster JA.** Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* 23: 255-264, e119, 2011.
88. **O' Mahony SM, Dinan TG, and Cryan JF.** The gut microbiota as a key regulator of visceral pain. *Pain* 158 Suppl 1: S19-S28, 2017.
89. **O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, and Quigley EM.** Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 128: 541-551, 2005.
90. **O'Mahony SM, Felice VD, Nally K, Savignac HM, Claesson MJ, Scully P, Woznicki J, Hyland NP, Shanahan F, Quigley EM, Marchesi JR, O'Toole PW, Dinan TG, and Cryan JF.**

- Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. *Neuroscience* 277: 885-901, 2014.
91. **Ochoa-Cortes F, Ramos-Lomas T, Miranda-Morales M, Spreadbury I, Ibeakanma C, Barajas-Lopez C, and Vanner S.** Bacterial cell products signal to mouse colonic nociceptive dorsal root ganglia neurons. *Am J Physiol Gastrointest Liver Physiol* 299: G723-G732, 2010.
 92. **Okumura T, Nozu T, Kumei S, Takakusaki K, Miyagishi S, and Ohhira M.** Involvement of the dopaminergic system in the central orexin-induced antinociceptive action against colonic distension in conscious rats. *Neuroscience letters* 605: 34-38, 2015.
 93. **Perez-Burgos A, Wang L, McVey Neufeld KA, Mao YK, Ahmadzai M, Janssen LJ, Stanis AM, Bienenstock J, and Kunze WA.** The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic *Lactobacillus reuteri* DSM 17938. *The Journal of physiology* 593: 3943-3957, 2015.
 94. **Pokusaeva K, Johnson C, Luk B, Uribe G, Fu Y, Oezguen N, Matsunami RK, Lugo M, Major A, Mori-Akiyama Y, Hollister EB, Dann SM, Shi XZ, Engler DA, Savidge T, and Versalovic J.** GABA-producing *Bifidobacterium dentium* modulates visceral sensitivity in the intestine. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 29, 2017.
 95. **Powley TL, Spaulding RA, and Haglof SA.** Vagal afferent innervation of the proximal gastrointestinal tract mucosa: chemoreceptor and mechanoreceptor architecture. *J Comp Neurol* 519: 644-660, 2011.
 96. **Pusceddu MM and Gareau MG.** Visceral pain: gut microbiota, a new hope? *J Biomed Sci* 25: 73, 2018.
 97. **Ramachandran R, Altier C, Oikonomopoulou K, and Hollenberg MD.** Proteinases, Their Extracellular Targets, and Inflammatory Signaling. *Pharmacological reviews* 68: 1110-1142, 2016.
 98. **Rea K, O'Mahony SM, Dinan TG, and Cryan JF.** The Role of the Gastrointestinal Microbiota in Visceral Pain. *Handbook of experimental pharmacology* 239: 269-287, 2017.
 99. **Reigstad CS, Salmonson CE, Rainey JF, 3rd, Szurszewski JH, Linden DR, Sonnenburg JL, Farrugia G, and Kashyap PC.** Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J* 29: 1395-1403, 2015.
 100. **Reijnders D, Goossens GH, Hermes GD, Neis EP, van der Beek CM, Most J, Holst JJ, Lenaerts K, Kootte RS, Nieuwdorp M, Groen AK, Olde Damink SW, Boekschoten MV, Smidt H, Zoetendal EG, Dejong CH, and Blaak EE.** Effects of Gut Microbiota Manipulation by Antibiotics on Host Metabolism in Obese Humans: A Randomized Double-Blind Placebo-Controlled Trial. *Cell Metab* 24: 63-74, 2016.
 101. **Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, and Gribble FM.** Glucose sensing in L cells: a primary cell study. *Cell metabolism* 8: 532-539, 2008.
 102. **Ringel-Kulka T, Goldsmith JR, Carroll IM, Barros SP, Palsson O, Jobin C, and Ringel Y.** *Lactobacillus acidophilus* NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study. *Alimentary pharmacology & therapeutics* 40: 200-207, 2014.
 103. **Roediger WE.** Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 21: 793-798, 1980.

104. **Rolland-Fourcade C, Denadai-Souza A, Cirillo C, Lopez C, Jaramillo JO, Desormeaux C, Cenac N, Motta JP, Larauche M, Tache Y, Berghe PV, Neunlist M, Coron E, Kirzin S, Portier G, Bonnet D, Alric L, Vanner S, Deraison C, and Vergnolle N.** Epithelial expression and function of trypsin-3 in irritable bowel syndrome. *Gut* 66: 1767-1778, 2017.
105. **Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M, Ouwehand A, Leyer G, Carcano D, Colombel JF, Ardid D, and Desreumaux P.** Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature medicine* 13: 35-37, 2007.
106. **Sander LE, Lorentz A, Sellge G, Coeffier M, Neipp M, Veres T, Frieling T, Meier PN, Manns MP, and Bischoff SC.** Selective expression of histamine receptors H1R, H2R, and H4R, but not H3R, in the human intestinal tract. *Gut* 55: 498-504, 2006.
107. **Savignac HM, Corona G, Mills H, Chen L, Spencer JP, Tzortzis G, and Burnet PW.** Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-D-aspartate receptor subunits and D-serine. *Neurochem Int* 63: 756-764, 2013.
108. **Selwyn FP, Csanaky IL, Zhang Y, and Klaassen CD.** Importance of Large Intestine in Regulating Bile Acids and Glucagon-Like Peptide-1 in Germ-Free Mice. *Drug Metab Dispos* 43: 1544-1556, 2015.
109. **Sessenwein JL, Baker CC, Pradhananga S, Maitland ME, Petrof EO, Allen-Vercos E, Noordhof C, Reed DE, Vanner SJ, and Lomax AE.** Protease-Mediated Suppression of DRG Neuron Excitability by Commensal Bacteria. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 37: 11758-11768, 2017.
110. **Shen S, Al-Thumairy HW, Hashmi F, and Qiao LY.** Regulation of transient receptor potential cation channel subfamily V1 protein synthesis by the phosphoinositide 3-kinase/Akt pathway in colonic hypersensitivity. *Experimental neurology* 295: 104-115, 2017.
111. **Smolinska S, Groeger D, Perez NR, Schiavi E, Ferstl R, Frei R, Konieczna P, Akdis CA, Jutel M, and O'Mahony L.** Histamine Receptor 2 is Required to Suppress Innate Immune Responses to Bacterial Ligands in Patients with Inflammatory Bowel Disease. *Inflammatory bowel diseases* 22: 1575-1586, 2016.
112. **Song Z, Xie W, Chen S, Strong JA, Print MS, Wang JI, Shareef AF, Ulrich-Lai YM, and Zhang JM.** High-fat diet increases pain behaviors in rats with or without obesity. *Sci Rep* 7: 10350, 2017.
113. **Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain JP, and Neunlist M.** Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. *Gastroenterology* 138: 1772-1782, 2010.
114. **Spinler JK, Sontakke A, Hollister EB, Venable SF, Oh PL, Balderas MA, Saulnier DM, Mistretta TA, Devaraj S, Walter J, Versalovic J, and Highlander SK.** From prediction to function using evolutionary genomics: human-specific ecotypes of Lactobacillus reuteri have diverse probiotic functions. *Genome Biol Evol* 6: 1772-1789, 2014.
115. **Stanisor OI, van Diest SA, Yu Z, Welting O, Bekkali N, Shi J, de Jonge WJ, Boeckxstaens GE, and van den Wijngaard RM.** Stress-induced visceral hypersensitivity in maternally separated rats can be reversed by peripherally restricted histamine-1-receptor antagonists. *PLoS one* 8: e66884, 2013.
116. **Steck N, Mueller K, Schemann M, and Haller D.** Bacterial proteases in IBD and IBS. *Gut* 61: 1610-1618, 2012.

117. **Steinert RE, Feinle-Bisset C, Asarian L, Horowitz M, Beglinger C, and Geary N.** Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. *Physiological reviews* 97: 411-463, 2017.
118. **Stenman LK, Waget A, Garret C, Briand F, Burcelin R, Sulpice T, and Lahtinen S.** Probiotic B420 and prebiotic polydextrose improve efficacy of antidiabetic drugs in mice. *Diabetol Metab Syndr* 7: 75, 2015.
119. **Strandwitz P.** Neurotransmitter modulation by the gut microbiota. *Brain research* 1693: 128-133, 2018.
120. **Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, and Koga Y.** Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *The Journal of physiology* 558: 263-275, 2004.
121. **Tarrerias AL, Millecamps M, Alloui A, Beaughard C, Kemeny JL, Bourdu S, Bommelaer G, Eschaliere A, Dapoigny M, and Ardid D.** Short-chain fatty acid enemas fail to decrease colonic hypersensitivity and inflammation in TNBS-induced colonic inflammation in rats. *Pain* 100: 91-97, 2002.
122. **Theodorou V, Ait Belgnaoui A, Agostini S, and Eutamene H.** Effect of commensals and probiotics on visceral sensitivity and pain in irritable bowel syndrome. *Gut microbes* 5: 430-436, 2014.
123. **Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, and Gribble FM.** Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 61: 364-371, 2012.
124. **Valdez-Morales E, Guerrero-Alba R, Ochoa-Cortes F, Benson J, Spreadbury I, Hurlbut D, Miranda-Morales M, Lomax AE, and Vanner S.** Release of endogenous opioids during a chronic IBD model suppresses the excitability of colonic DRG neurons. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 25: 39-46 e34, 2013.
125. **Vanhoutvin SA, Troost FJ, Kilkens TO, Lindsey PJ, Hamer HM, Jonkers DM, Venema K, and Brummer RJ.** The effects of butyrate enemas on visceral perception in healthy volunteers. *Neurogastroenterol Motil* 21: 952-e976, 2009.
126. **Verdu EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P, Jackson W, Mao Y, Wang L, Rochat F, and Collins SM.** Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 55: 182-190, 2006.
127. **Vergnolle N.** Protease-activated receptors as drug targets in inflammation and pain. *Pharmacology & therapeutics* 123: 292-309, 2009.
128. **Vergnolle N.** Protease inhibition as new therapeutic strategy for GI diseases. *Gut* 65: 1215-1224, 2016.
129. **Wall R, Cryan JF, Ross RP, Fitzgerald GF, Dinan TG, and Stanton C.** Bacterial neuroactive compounds produced by psychobiotics. *Adv Exp Med Biol* 817: 221-239, 2014.
130. **Wang FB and Powley TL.** Topographic inventories of vagal afferents in gastrointestinal muscle. *J Comp Neurol* 421: 302-324, 2000.
131. **Wu RY, Pasyk M, Wang B, Forsythe P, Bienenstock J, Mao YK, Sharma P, Stanis AM, and Kunze WA.** Spatiotemporal maps reveal regional differences in the effects on gut motility for *Lactobacillus reuteri* and *rhamnosus* strains. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society* 25: e205-214, 2013.

132. **Xia CM, Gulick MA, Yu SJ, Grider JR, Murthy KS, Kuemmerle JF, Akbarali HI, and Qiao LY.** Up-regulation of brain-derived neurotrophic factor in primary afferent pathway regulates colon-to-bladder cross-sensitization in rat. *Journal of neuroinflammation* 9: 30, 2012.
133. **Xu D, Wu X, Grabauskas G, and Owyang C.** Butyrate-induced colonic hypersensitivity is mediated by mitogen-activated protein kinase activation in rat dorsal root ganglia. *Gut* 62: 1466-1474, 2013.
134. **Xu XJ, Zhang YL, Liu L, Pan L, and Yao SK.** Increased expression of nerve growth factor correlates with visceral hypersensitivity and impaired gut barrier function in diarrhoea-predominant irritable bowel syndrome: a preliminary explorative study. *Alimentary pharmacology & therapeutics* 45: 100-114, 2017.
135. **Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, and Hsiao EY.** Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161: 264-276, 2015.
136. **Zhao P, Lieu T, Barlow N, Metcalf M, Veldhuis NA, Jensen DD, Kocan M, Sostegni S, Haerteis S, Baraznenok V, Henderson I, Lindstrom E, Guerrero-Alba R, Valdez-Morales EE, Liedtke W, McIntyre P, Vanner SJ, Korbmacher C, and Bunnett NW.** Cathepsin S causes inflammatory pain via biased agonism of PAR2 and TRPV4. *The Journal of biological chemistry* 289: 27215-27234, 2014.
137. **Zhou SY, Gilliland M, 3rd, Wu X, Leelasinjaroen P, Zhang G, Zhou H, Ye B, Lu Y, and Owyang C.** FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. *J Clin Invest* 128: 267-280, 2018.
138. **Zurowski D, Nowak L, Wordliczek J, Dobrogowski J, and Thor PJ.** Effects of vagus nerve stimulation in visceral pain model. *Folia Med Cracov* 52: 57-69, 2012.

791 Table 1: *In vivo* studies of the effects of probiotics on visceral pain.

Probiotic strain	Reference	Main finding	Proposed mechanism
<i>Lactobacillus rhamnosus</i> and/or prebiotics polydextrose/galactooligosaccharide	(65)	Neonatal zymosan-treated rats treated with probiotic did not exhibit visceral hyperalgesia in response to CRD in adulthood	Altered CNS neurotransmitters
<i>Lactobacillus reuteri</i>	(93)	Inhibited the bradycardia induced by painful gastric distension in rats	TRPV1 modulation
<i>Lactobacillus paracasei</i>	(126)	Normalized visceral sensitivity to CRD in antibiotic treated mice in mice	Immunomodulation
	(46)	Prevented the maternal deprivation increased visceral sensitivity in response to CRD in rats	Epithelial barrier regulation
<i>Lactobacillus acidophilus</i>	(105)	Normalized visceral pain responses to CRD in mice and rats	Altered epithelial expression of opioid and cannabinoid receptors
	(102)	Reduced bloating symptoms in patients with functional bowel diseases experiencing abdominal pain in females	Modulated μ -opioid receptor expression and activity
<i>Lactobacillus farciminis</i>	(3)	Reversed visceral hypersensitivity induced by partial restraint stress (PRS) in rats	Epithelial barrier regulation
	(2)	Inhibited Fos protein expression at spinal and supraspinal levels as a marker of visceral pain in response to CRD in rats after PRS	None specified
<i>Bifidobacterium infantis</i>	(64)	Reversed post-inflammatory (TNBS) visceral hypersensitivity in rats	Immunomodulation
<i>Bifidobacterium lactis</i>	(1)	Inhibited PRS-induced visceral hypersensitivity in rats	Epithelial barrier regulation
<i>Bifidobacterium longum</i> and <i>Lactobacillus helveticus</i>	(4)	Reduced chronic stress-induced visceral hypersensitivity in mice	Regulation of hypothalamic-pituitary-adrenal axis

<i>Bifidobacterium infantis</i> , <i>Lactobacillus salivarius</i> , <i>Bifidobacterium breve</i>	(79)	Reduced CRD-induced visceral pain behaviours in rats	None specified
<i>Bifidobacterium infantis</i> or <i>Lactobacillus salivarius</i>	(89)	<i>Bifidobacterium infantis</i> decreased visceral pain more than <i>Lactobacillus salivarius</i> or placebo in IBS patients	Immunomodulation
<i>Lactibiane Tolerance®</i> : <i>Lactobacillus acidophilus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus salivarius</i> <i>Bifidobacterium lactis</i>	(86)	Reversed visceral hypersensitivity induced by water-avoidance stress or IBS fecal supernatant administration in mice	Epithelial barrier regulation
<i>VSL#3 Bifidobacterium (B. longum, B. infantis and B. breve); Lactobacillus (L. acidophilus, L. casei, L. delbrueckii ssp. bulgaricus and L. plantarum); and Streptococcus salivarius ssp. Thermophilus</i>	(44)	Early life administration of VSL#3 reduced visceral pain perception in a model of IBS in rats	Altered colonic expression of genes influencing pain and inflammation
	(36)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Epithelial barrier regulation
	(71)	Decreases acetic-acid-induced visceral hypersensitivity in rats	Immunomodulation
<i>Faecalibacterium prausnitzii</i>	(81)	Decreased colonic hypersensitivity induced by either NMS in mice or partial restraint stress in rats	Epithelial barrier regulation

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Figure 1: Microbial modulation of visceral afferent pathways

The figure illustrates potential mechanisms by which microbes in the gut lumen could modify afferent signaling from the gut to the central nervous system. The microbiota can affect the sensitivity of peripheral pain pathways by direct effects on the peripheral terminals of DRG neurons or indirectly by changing mediator release from enteroendocrine cells, immune cells or enterocytes. NTS: nucleus tractus solitarius, DRG: dorsal root ganglion, ENS: enteric nervous system, ECC: enterochromaffin cell, TLRs: Toll-like receptors.

Figure 1

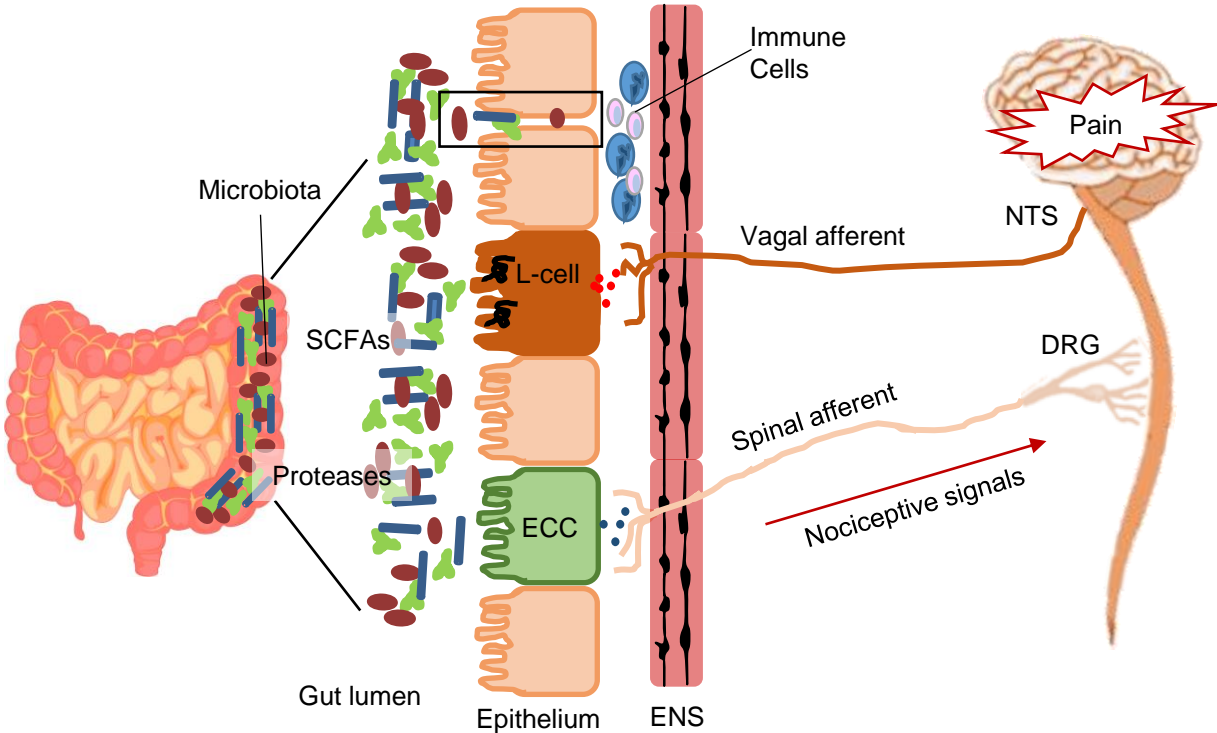


Figure 1

